AMENDMENTS TO THE SPECIFICATION

As per the Examiner's request, Applicant is providing herewith a substitute specification in both Marked-up and Clean versions. The following are the amendments appearing in the substitute specification:

The paragraph on page 1, lines 2-6, has been replaced with the following paragraph:

Benefit of priority under 35 U.S.C. §119(e) is claimed to U.S. patent 6,800,728, patent application No.: 09/815,978, filed March 22, 2001 entitled "HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL CROSSLINKING REAGENTS" which claims priority to U.S. provisional patent application No. 60/191,186, filed March 22, 2000, to Schwartz, entitled "NOVEL HYDRAZINE-BASED AND CARBONYL-BASED BIFUNCTIONAL CROSSLINKING REAGENTS." The disclosures of the above-referenced applications are incorporated herein in their entirety.

The paragraph on page 1, lines 11-17, has been replaced with the following paragraph:

Methods to crosslink biomolecules such as proteins, oligonucleotides and carbohydrates to each other, to radioactive and non-radioactive metal chelates, to drugs and to surfaces [[has]] have allowed development of both in vitro and in vivo diagnostic assays as well as in vivo therapies. A wide variety of methods have been developed and reviewed (Greg T. Hermanson, Bioconjugate Techniques, Academic Press).

The paragraph on page 4, lines 6 through 22, has been replaced with the following paragraph:

[[R^{11}]], R^{10}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, R^{19} and R^{20} can be substituted with one or more substituents each independently selected from Z, wherein Z is selected from alkyl, alkenyl, alkynyl, aryl, cycloalkyl, cycloalkenyl, hydroxy, $S(O)_hR^{30}$, $NR^{30}R^{31}$, $COOR^{30}$, COR^{30}, $CONR^{30}R^{31}$, $OC(O)NR^{30}R^{31}$, $N(R^{30})C(O)R^{31}$, alkoxy, aryloxy, heteroaryl, heterocyclyl, heteroaryloxy, heterocyclyloxy, aralkyl, aralkenyl, aralkynyl, heteroaralkyl, heteroaralkenyl,

heteroaralkynyl, aralkoxy, heteroaralkoxy, alkoxycarbonyl, carbamoyl, thiocarbamoyl, alkoxycarbonyl, carboxyaryl, halo, pseudohalo, haloalkyl and carboxamido; h is 0, 1 or 2; and R^{30} and R^{31} are each independently selected from among hydrogen, halo, pseudohalo, cyano, azido, nitro, trialkylsilyl, dialkylarylsilyl, alkyldiarylsilyl, triarylsilyl, alkyl, alkenyl, alkynyl, haloalkyl, haloalkoxy, aryl, aralkyl, aralkenyl, aralkynyl, heteroaryl, heteroaralkyl, heteroaralkenyl, heteroaralkynyl, heterocyclyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, hydroxy, alkoxy, aryloxy, aralkoxy, heteroaralkoxy, amino, amido, alkylamino, dialkylamino, alkylarylamino, diarylamino and arylamino.

The paragraph on page 9, line 26 to page 10 line 24, has been replaced with the following paragraph:

As used herein, a derivative of a compound is ~~includes~~ a salt, ester, enol ether, enol ester, solvate or hydrate thereof that can be prepared by those of skill in this art using known methods for such derivatization. Salts ~~include, but are not limited to,~~ may be amine salts, such as but not limited to N,N'- dibenzylethylenediamine, chloroprocaine, choline, ammonia, diethanolamine and other hydroxyalkylamines, ethylenediamine, N-methylglucamine, procaine, N-benzylphenethylamine, 1-para-chlorobenzyl-2-pyrrolidin-1'-ylmethylbenzimidazole, diethylamine and other alkylamines, piperazine and tris(hydroxymethyl)aminomethane; alkali metal salts, such as but not limited to lithium, potassium and sodium; alkali earth metal salts, such as but not limited to barium, calcium and magnesium; transition metal salts, such as but not limited to zinc; and other metal salts, such as but not limited to sodium hydrogen phosphate and disodium phosphate; and also, ~~including~~ but not limited to, salts of mineral acids, such as but not limited to hydrochlorides and sulfates; and salts of organic acids, such as but not limited to acetates, lactates, malates, tartrates, citrates, ascorbates, succinates, butyrates, valerates and fumarates. Esters ~~include~~ maybe, but are not limited to, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl and heterocyclyl esters of acidic groups, [including] such as, but not limited to, carboxylic acids, phosphoric acids, phosphinic acids, sulfonic acids, sulfinic acids and boronic acids. Enol ethers may be, but are not limited to, derivatives of formula C=C(OR) where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl [[ar]] and heterocyclyl. Enol esters ~~include~~ may be, but are not limited to, derivatives

of formula C=C(OC(O)R) where R is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, aralkyl, heteroaralkyl, cycloalkyl [[ar]] and heterocyclyl. Solvates and hydrates are complexes of a compound with one or more solvent or water molecule, preferably 1 to about 100, more preferably 1 to about 10, most preferably one to about 2, 3 or 4, solvent or water molecules.

The paragraph on page 11, line 1 through 16, has been replaced with the following paragraph:

As used herein, alkyl, alkenyl and alkynyl carbon chains, if not specified, contain from 1 to 20 carbons, preferably 1 to 16 carbons, and are straight or branched. Alkenyl carbon chains of from 2 to 20 carbons preferably contain 1 to 8 double bonds, and the alkenyl carbon chains of 1 to 16 carbons preferably contain 1 to 5 double bonds. Alkynyl carbon chains of from 2 to 20 carbons preferably contain 1 to 8 triple bonds, and the alkynyl carbon chains of 2 to 16 carbons preferably contain 1 to 5 triple bonds. Exemplary alkyl, alkenyl and alkynyl groups herein include, but are not limited to, methyl, ethyl, propyl, isopropyl, isobutyl, n-butyl, sec-butyl, tert-butyl, isopentyl, neopentyl, tert-~~penytyl~~ pentyl and isohexyl. The alkyl, alkenyl and alkynyl groups, unless otherwise specified, can be optionally substituted, with one or more groups, preferably alkyl group substituents that can be the same or different. As used herein, lower alkyl, lower alkenyl, and lower alkynyl refer to carbon chains having less than about 6 carbons. As used herein, "alk(en)(yn)yl" refers to an alkyl group containing at least one double bond and at least one triple bond.

The paragraph on page 11, line 26 through page 12 line 12, has been replaced with the following paragraph:

As used herein, an "aryl group substituent" includes alkyl, cyclo-alkyl, cycloalkylalkyl, aryl, heteroaryl optionally substituted with 1 or more, preferably 1 to 3, substituents selected from halo, ~~halo-alkyl~~ haloalkyl and alkyl, aralkyl, heteroaralkyl, alkenyl containing 1 to 2 double bonds, alkynyl containing 1 to 2 triple bonds, alk(en)(yn)yl groups, halo, pseudohalo, cyano, hydroxy, ~~halo-alkyl~~ haloalkyl and polyhaloalkyl, preferably halo lower alkyl, especially trifluoromethyl, formyl, alkylcarbonyl, arylcarbonyl that is optionally substituted with 1 or more, preferably 1 to 3, substituents selected from halo, haloalkyl and alkyl, heteroarylcarbonyl,

carboxy, alkoxycarbonyl, aryloxycarbonyl, aminocarbonyl, alkylaminocar-bonyl, dialkylaminocarbonyl, arylaminocarbonyl, diarylaminocarbonyl, aralkylaminocarbonyl, alkoxy, aryloxy, perfluoroalkoxy, alkenyloxy, alkynyloxy, arylalkoxy, aminoalkyl, alkylaminoalkyl, dialkylaminoalkyl, arylaminoalkyl, amino, alkylamino, dialkylamino, arylamino, alkylarylamino, alkylcarbonylamino, arylcarbonylamino, azido, nitro, mercapto, alkylthio, arylthio, perfluoroalkylthio, thiocyano, isothiocyano, alkylsulfinyl, alkylsulfonyl, arylsulfinyl, arylsulfonyl, aminosulfonyl, alkylaminosulfonyl, dialkylaminosulfonyl and arylaminosulfonyl.

The paragraph on page 12, line 31 through page 13 line 9, has been replaced with the following paragraph:

As used herein, "heteroaryl" refers to a monocyclic or multicyclic ring system, preferably of about 5 to about 15 members where one or more, more preferably 1 to 3 of the atoms in the ring system is a heteroatom, that is, an element other than carbon, for example, nitrogen, oxygen and sulfur atoms. The heteroaryl can be optionally substituted with one or more, preferably 1 to 3, aryl group substituents. The heteroaryl group can be optionally fused to a benzene ring. Exemplary heteroaryl groups include, for example, furyl, imidazolyl ~~imidazinlyl~~, pyrrolidinyl, pyrimidinyl, tetrazolyl, thienyl, pyridyl, pyrrolyl, N-methylpyrrolyl, quinolinyl and isoquinolinyl, with pyridyl and quinolinyl being preferred.

The paragraph on page 19, line 29 through page 20 line 2, has been replaced with the following paragraph:

As used herein, the abbreviations for any protective groups, amino acids and other compounds, are, unless indicated otherwise, in accord with their common usage, recognized abbreviations, or the IUPAC-IUB Commission on Biochemical Nomenclature (see, Biochem. ~~1972~~ 1972, 11, 942).

The paragraph on page 24, lines 20-27, has been replaced with the following paragraph:

In another embodiment, the bifunctional hydrazide reagents provided herein form acid cleavable hydrazones. These reagents are advantageous as [[the]] they can be used to modify biomolecules or carriers such as polymers in a single step. These modified aliphatic hydrazide biomolecules or carriers can subsequently reacted with carbonyl containing biomolecules, drug or other therapeutic or diagnostic reagent to readily form a hydrazone that can be cleaved following exposure to mild aqueous acid conditions at pH <5.

The paragraph on page 33, lines 10 through 24, has been replaced with the following paragraph:

The development of both DNA-based and protein microarrays has led to a revolution in biotechnology. These microarrays are based on immobilization of tens to tens of thousand biomolecules on solid surfaces. Silica based surfaces such as glass slides and silica chips have been the surface of choice to prepare microarrays. The immobilization of biomolecules requires attachment of the biomolecules via covalent or non-covalent, i.e., electrostatic, interactions. Glass slides modified to incorporate amino or aldehyde groups are commercially available (www.arrayit.com, Telechem, Inc., ~~someplace~~Sunnyvale, CA and www.cel-1.com, Cel Associates, Houston, TX). Protocols to immobilize oligonucleotides or polynucleotides require the use of strong chemical conditions such as sodium borohydride or crosslinking conditions such as photolysis. These methods are inefficient and cause direct modification of the oligonucleotide leading to reduced affinity towards its complementary target."

The paragraphs on page 38, lines 8 through 15, have been replaced with the following paragraphs:

Bifunctional carbazides or thiocarbazides may be prepared by treatment of a hydrazine with phosgene or thiophosgene, respectively, in the presence of base followed by isolation of the iso(thio)cyanate. Addition of hydrazine yields the desired carbazide or thiocarbazide respectively.

Bifunctional semicarbazides or thiosemicarbazides may be prepared by treatment of an amine with phosgene or thiophosgene, respectively, in the presence of base followed by isolation of the iso(thio)cyanate. Addition of hydrazine yields the desired semicarbazide ~~carbazide~~ or thiosemicarbazide ~~thiocarbazide~~ respectively.

The following paragraphs on page 41, line 18 through page 42 line 4, have been replaced with the following paragraphs:

To a solution of this residue in [[THF]]DMF is added t-butyl carbazate (1.0 equivalent; Aldrich Chemical Company, Milwaukee, WI) in [[THF]]DMF. The reaction mixture is stirred at room temperature for 2 hours. The solvent is removed under reduced pressure and the residue is partitioned between ethyl acetate and 5% aqueous citric acid. The organic phase is washed with brine, dried over magnesium sulfate, filtered and concentrated to give 4-(tert-butoxycarbonylthiosemi-carbazidomethyl)cyclohexane carboxylic acid.

This compound (1.0 equivalent) is dissolved in [[THF]]DMF and N- hydroxysuccinimide (1.0 equivalent) is added followed by the dropwise addition of a solution of dicyclohexylcarbodiimide (1.0 equivalents) in [[THF]]DMF. The reaction mixture is stirred at room temperature for 4 hours. The dicyclohexylurea (DCU) precipitate byproduct is removed by filtration and the filtrate is concentrated to dryness. The residue is partitioned between ethyl acetate and 5% aqueous citric acid. The organic phase is washed with brine, dried over magnesium sulfate, filtered and concentrated to give succinimidyl 4-(tert-butoxycarbonylthiosemi-carbazido-methyl)cyclohexane carboxylate.

The paragraph on page 42, lines 21 through 31, has been replaced with the following paragraph:

This hydrazone (1.0 equivalent) was suspended in DMF and N-hydroxysuccinimide (NHS)(1.0 equivalent) was added and followed by the addition of a solution of DCC (1.0 equivalent) in DMF ~~was added~~. The reaction mixture was stirred at room temperature for 16 hours. The heterogeneous reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was dissolved in a minimum amount of ethyl acetate and hexanes were added to turbidity. A pale yellow precipitate formed that was isolated by filtration to give

the desired compound with an approximate yield of 33%. PMR (DMSO-d_6) δ 1.99 (s, 3H), 2.00 (s, _3H), 3.32 (s, 4H), 7.17 (D, 1H), 8.12 (dd, 1H), 8.76 (d, 1H), 10.39 (s, 1H).

The paragraph on page 43, lines 12 through 18, has been replaced with the following paragraph:

To a solution of the resulting compound (1 mmol) in THF is added maleic anhydride (1 mmol), the reaction mixture is stirred at room temperature and acetic anhydride (1 mmol) and triethylamine (1 mmol) [[is]]are added. Following stirring at room temperature for 16 hours, the solvent is removed under reduced pressure, and the residue is chromatographed on silica gel using ethyl acetate as eluant. The fractions containing product are pooled and concentrated.

The paragraph on page 43, line 26 through page 44 line 3, has been replaced with the following paragraph:

To a suspension of proline (1 mmol) in THF is added triethylamine (2.5 mmol) followed by the dropwise addition of a solution of thiophosgene (1.1 mmol). The reaction mixture is stirred at ambient temperature for 4 hours followed by cooling the reaction mixture to 0°C and the dropwise addition of a solution of t-butyl carbazate (1.1 mL). The reaction mixture is stirred at 0°C for 1 hour and at room temperature for 2 hours. The solvent is removed under reduced pressure and the residue is chromatographed on silica gel using methylene chloride/~~methonal~~ methanol (9/1) as eluant. The fractions containing product are pooled and concentrated.

The paragraph on page 46, lines 5-12, has been replaced with the following paragraph:

Amino-modified 96 well plates (Costar or Corning) are modified with succinimidyl 4-formylbenzoate (SFB) as follows. A fresh solution of SFB (1.25 mL of 10 mg/mL) in DMSO is prepared. This solution is diluted into phosphate buffered saline (PBS)(0.1 M phosphate, 0.15 M NaCl, pH 7.4: 100 mL). To each well is added 200 μL of the SFB/PBS solution and the wells are incubated at room temperature for 4 hours. The wells are washed three times with PBS/0.5% ~~tween~~ Tween®. The wells are dried and are ready for protein conjugation.

The paragraph on page 46, lines 15-24, has been replaced with the following paragraph:

Amino-modified 96 well plates (Costar or Corning) are modified with succinimidyl acetone nicotinic acid hydrazone (SANH) as follows. A fresh solution of SANH (1.25 mL of 10 mg/mL) in DMSO is prepared. This solution is diluted into PBS (0.1 M ~~phosphate~~ phosphate, 0.15 M NaCl, pH 7.4: 100 mL). To each well was added 200 µL of the [[SFB]]SANH/PBS solution and the wells were incubated at room temperature for 4 hours. The wells are washed with water and then treated with 0.1 M acetate, pH 4.7 (200 uL) for 2 hours. The wells were washed three times with PBS/0.5% Tween®. The wells were dried and are ready for conjugation to molecules possessing carbonyl moieties.

The paragraph on page 46, line 28 through page 47 line 8, has been replaced with the following paragraph:

A 5 mg/mL solution of bovine serum albumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4 and 2 mM EDTA) (200 µL; 1 mg protein) is prepared. A solution of succinimidyl 4-semicarbazidylbenzoate hydrochloride (SSCH; 3.5 mg) in DMF (100 µL) is prepared. To the protein solution is added the SSCH/DMF solution (30 equivalents). The reaction mixture is incubated at room temperature for 4 hours. The modified protein is isolated by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with conjugation buffer (3 X 400 µL). The purified protein is quantitated for protein concentration (BCA assay) and for hydrazine modification level by addition of 0.2 [[Mm]]mM 2-p-nitrobenzaldehyde in PBS pH 7.4 and measuring the absorbance at 380nm (extinction coefficient 22,600).

The paragraph on page 47, line 28 through page 48 line 10, has been replaced with the following paragraph:

A 5 mg/mL solution of ovalbumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4) and 2 mM EDTA (200 µL; 1 mg protein) was prepared. A solution of succinimidyl 6-

hydrazinonicotinate acetone hydrazone (SANH)(EXAMPLE 2)(2 mg) in DMF (50 μL) is prepared. To the protein solution was added the SANH/DMF solution (15 [[eq.]] equivalents. The reaction mixture was incubated at room temperature for 4 [[hrs]]hours. The modified protein was isolated and buffer exchanged by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with 0.1 M MES, 0.9% NaCl, pH 4.7 (3 X 400 μL). The purified protein was quantified for protein concentration (BCA assay; Pierce Chemical Co., Rockford, IL) and for hydrazine modification level by incubation of an aliquot of protein in a 0.5 mM 4-nitrobenzaldehyde in 0.1 M MES, 0.9% NaCl, pH 4.7 and measuring the absorbance at 360 nm (molar extinction coefficient 22,000).

The paragraph on page 48, lines 14-22, has been replaced with the following paragraph:

Aldehyde-modified IgG (EXAMPLE 10) in MES (1 mg; 0.200 μL of a 2.5 mg/mL solution), was added to a solution of hydrazine-modified ovalbumin (EXAMPLE 11, 1 mL; 0.200 μL of a 5 mg/mL solution) and the reaction mixture was incubated at room temperature for 4 hours. The reaction mixture was analyzed by PAGE gel (coomassie blue development) that demonstrated presence of a high molecular weight product and <5% unreacted aldehyde-modified IgG and <10% unreacted hydrazine-modified ovalbumin. The level of conjugation is quantified by measuring the absorbance at 360 nm.

The paragraph on page 48, line 25 through page 49 line 3, has been replaced with the following paragraph:

A 5 mg/mL solution of ovalbumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4) and 2 mM EDTA (200 μL; 1 mg protein) was prepared. A solution of succinimidyl 4-thiosemicarbazidylbenzoate hydrochloride (STBH)(2 mg) in DMF (50 μL) is prepared. To the protein solution was added the STBH/DMF solution (15 [[eq.]] equivalents. The reaction mixture was incubated at room temperature for 4 [[hrs]]hours. The modified protein was isolated and buffer exchanged by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with 0.1 M MES, 0.9% NaCl, pH 4.7 (3 X 400 μL). The purified protein was quantified for protein concentration (BCA assay; Pierce Chemical Co., Rockford, IL).

The paragraph on page 49, lines 7-11, has been replaced with the following paragraph:

The thiosemicarbazide protein prepared in EXAMPLE 13 was reacted with aldehyde-modified protein in an identical manner as described for the hydrazine-modified protein in EXAMPLE 12. Analysis by PAGE gel demonstrated similar efficiency as conjugation [[as]]observed in EXAMPLE 12.

The paragraph on page 49, lines 14-23, has been replaced with the following paragraph:

A 5 mg/mL solution of ovalbumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4) and 2 mM EDTA (200 μL; 1 mg protein) was prepared. A solution of succinimidyl 4-hydrazidoterephalate hydrochloride (SHTH)(2 mg) in DMF (50 μL) is prepared. To the protein solution was added the SHTH/DMF solution (15 [[eq.]] equivalents. The reaction mixture was incubated at room temperature for 4 [[hrs]]hours. The modified protein was isolated and buffer exchanged by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with 0.1 M MES, 0.9% NaCl, pH 4.7 (3 X 400 μL). The purified protein was quantified for protein concentration (BCA assay; Pierce Chemical Co., Rockford, IL).

The paragraph on page 49, lines 25 through 31, has been replaced with the following paragraph:

Conjugation of a hydrazine-modified protein to [[a]]an aldehyde-modified protein

The hydrazide-modified protein prepared in EXAMPLE 15 was reacted with aldehyde-modified protein in an identical manner as described for the hydrazine-modified protein in EXAMPLE 12. Analysis by PAGE gel demonstrated similar efficiency as conjugation [[as]] observed in EXAMPLE 12.

The paragraph on page 51, lines 17-22, has been replaced with the following paragraph:

Periodate-oxidized horseradish peroxidase (Pierce Chemical Co., Rockford, IL) is diluted to the desired concentration and added to 96- 3456 well plates that had been modified to possess

hydrazino groups as described above in EXAMPLE 9. The antibody solution is allowed to incubate for 2-18 [[hrs]]hours followed by removal of the solution and washing with 0.5% ~~tween~~ Tween® solution (twice) and buffer (twice).

The paragraph on page 51 line 28 to page 52 line 15, has been replaced with the following paragraph:

A 25-mer phosphodiester oligonucleotide modified to incorporate a C6-aminolinker (Glen Research amino-C6 amidite) was prepared (5'-NH_2-$(CH_2)_6$-ttt ttt tag cct aac tga tgc cat g-3' (SEQ ID NO.: 1); MW 7791 g/mol, 229.5 OD/μmol; TriLink BioTechnologies, Inc., San Diego, CA). The oligonucleotide was dissolved in conjugation buffer (100 mM phosphate, 150 mM sodium chloride, pH 7.4) to a concentration of 0.92 OD/μL. To a solution of oligonucleotide (64 μL; 2 mg) was added DMF (32 μL). A solution of SANH (EXAMPLE 2; 3.8 mg) in DMF (100 μL) was prepared. An aliquot of the SANH/DMF solution (18.8 μL; 10 equivalents) was added to the oligonucleotide solution and the reaction allowed to incubate at room temperature overnight. The reaction was monitored by C18 RP-HPLC (solution A: 50 mM triethylammonium acetate, solution B: acetonitrile- gradient 0-50% A over 30 min; 50-80% over 10 min; 80-0% over 5 min). The hydrazine-modified oligonucletide was deprotected and purified using a Millipore 5K MWCO ultrafree diafiltration device by diluting the reaction mixture with 100 mM acetate, pH 4.7 and concentrating in the diafiltration device. The retentate was further washed with buffer (2 X 400 μL). The oligonucleotide was quantified by A260 assay and the hydrazine incorporation was determined using the p- nitrobenzaldehyde assay described in EXAMPLE 9.

The paragraph on page 53, line 21 through page 54 line 8, has been replaced with the following paragraph:

As shown in FIGURE 9, to a solution of 6-chloronicotinic acid (1 equivalent) in 80% aqueous ethanol is added hydroxylamine (500 equivalents) and the solution is refluxed for 16 hours. The reaction mixture is concentrated to dryness and dissolved in water. The solution cooled in an ice bath and acidified with concentrated hydrochloric acid until a precipitate forms, pH approximately 5.0. The solids are isolated, redissolved ~~redissolve~~ in water and the pH of the

solution raised to 7.5 with base. Dioxane (1 volume) is added to the solution followed by the dropwise addition of di-t-butyl dicarbonate (5 equivalents; Aldrich Chemical Co.). The reaction mixture is stirred at room temperature for 4 hours and the dioxane removed on the rotavap. The residue is chromatographed on silica to isolate the desired BOC acid. The acid (1 ~~equiv~~equivalent) is dissolved in DMF and treated with NHS (1 equiv) followed by the dropwise addition of DCC (1 ~~equiv~~equivalent) in DMF. The reaction mixture is stirred at room temperature for 4 hours and the solids removed by filtration and the filtrate concentrated to dryness and resuspended in ethyl acetate. Further precipitate is removed by filtration and the filtrate concentrated to dryness and the desired BOC succinimidyl ester is isolated by silica gel chromatography.

The paragraph on page 54, lines 20-29, has been replaced with the following paragraph:

A 5 mg/mL solution of ovalbumin in PBS (100 mM phosphate, 150 mM NaCl, pH 7.4) and 2 mM EDTA (200 µL; 1 mg protein) is prepared. A solution of succinimidyl aminooxyacetate hydrochloride (SAAH)(2 mg) in DMF (50 µL) is prepared. To the protein solution is added the SAAH/DMF solution (15 [[eq.]] equivalents). The reaction mixture is incubated at room temperature for 4 [[hrs]]hours. The modified protein is isolated and buffer exchanged by placing the reaction mixture in a 30K ultra-free centrifugation device and washing three times with 0.1 M MES, 0.9% NaCl, pH 4.7 (3 X 400 µL). The purified protein is quantified for protein concentration (BCA assay; Pierce Chemical Co., Rockford, IL).

The paragraph on page 55, lines 3 through 20, has been replaced with the following paragraph:

A solution of poly-l-lysine (10 mg; Sigma Chemicals, St. Louis, MO; cat. #P-7890) was dissolved in conjugation buffer, 0.1 M phosphate, 0.15 M NaCl, pH 7.4 (1 mL). A solution of succinimidyl 6-hydrazinonicotinate acetone hydrazone (SANH; 1.3 mg) was dissolved in DMSO (13 µL). To two poly-l-lysine aliquots (200 µL) were added the SANH/DMSO solution (2.85 µL (10 equivalents) and 5.7 µL (20 equivalents)). The reaction mixtures were vortexed and incubated at room temperature for 2 [[h]]hours. The modified polymer was isolated by gel filtration on a NAP-25 column ~~Pharamae~~pre-equilibrated with 0.1 M MES, 0.9% NaCl, pH 4.7

buffer. Fractions (1 mL) were collected and analyzed by UV (A260). Fractions containing UV active product were combined to yield the desired product. The product was analyzed colorimetrically for hydrazine content by dissolving an aliquot (2 μL) in a 0.5 mM solution of p-nitrobenzaldehyde (98 μL) and incubating at 37°C for 1 [[h]]hour followed by taking A390 readings (extinction coefficient 22000). The HyNic:poly-l-lysine polymer was used directly in the conjugation step. The amine/hydrazine content was determined using the TNBSA assay (trinitrobenzenesulfonic acid; Pierce Chemical, Inc., Rockville, IL).

The paragraph on page 55, lines 24-30, has been replaced with the following paragraph:

A solution of a bacterial polysaccharide that possesses unsaturation in its lipids (from ATCC; 10 mg/mL) in water is treated with 10 mM sodium periodate (1/10 volume to make the solution 1 mM in periodate) and incubated at room temperature for 30 [[min]]minutes. The reaction mixture is passed through a sephadex G-25 column pre-equilibrated with water to remove small molecule impurities. The polysaccharide ~~combining~~ fractions are combined and concentrated to 5 mg/mL.